Three-dimensional Engineered Microenvironments to Study Stem Cell Niches *in vitro*

Milan Manchandia
Biochemistry 118Q
March 16, 2009

Stem cells are unique cells in that they posses the quality of maintaining the undifferentiated state through self-renewal and the capacity to differentiate into specialized cell types [1]. The two broad types of stem cells are pluripotent embryonic stem cells that are isolated from the inner cell mass of the blastocyst and give rise to all three primary germ layers and adult stem cells that act as a repair system for the body and maintain normal turnover of regenerative organs by replenishing specialized cells [1,2]. Stem cell therapy is a form of regenerative medicine in which adult of embryonic stem cells are used to repair damaged tissue or treat diseases [1]. However, due to the controversial destruction of human embryos in obtaining embryonic stem cells, stem cell therapy over the past decade has been severely limited. Therefore, current therapies in medicine mostly involve prevention, manipulation, and control of diseases through chemical or biological molecules [2]. However, a few stem cell therapies have been established in the clinical setting, as bone marrow transplantation has been used over the past few decades to treat certain types of leukemia [1,3]. In this case, hematopoietic stem cells from a healthy donor are injected into the irradiated bones of the leukemia patient where they will produce healthy leukocytes [1,3]. Furthermore, in 2005, a trial at Queen Victoria Hospital made use of stem cells to redevelop the cornea, which restored eyesight to 40 people [4]. Recently, doctors in Spain were able to carry out the world's first tissue-engineered whole organ transplant by using the patient's own stem cells to reconstruct a whole bronchial tube [5]. This successful transplantation sheds hope on the future therapeutic potential of stem cells in that a patient's own stem cells can be used to reconstruct damaged tissues and organs without risking the chance of donor rejection and need for immunosuppressive drugs. Moreover, Yamanaka and Takahashi's finding of the specific transcription factors Oct-3/4, SOX2, c-Myc, Klf4, and NANOG needed to reprogram human skin cells into induced pluripotent stem cells (IPSCs) [6]

was a major breakthrough by providing an ethical source of pluripotent stem cells that could potentially be differentiated into any tissue layer. These findings greatly support the notion that the future of stem cell therapy will involve the differentiation of one's own IPSCs into cells of a specific tissue layer, which would then be transplanted into the damaged tissue in the body. This type of stem cell therapy has broader implications in current stem cell research of stroke and brain damage, cancer, spinal cord injury, Parkinson's disease, Huntington's disease, heart reconstruction, and diabetes mellitus [1].

The true potential of pluripotent stem cells comes from their ability to differentiate into cells that give rise to all three germ layers. The proliferation and differentiation of stem cells are due to specific environmental regulatory signals and intrinsic programs that maintain stem cell properties [7]. This physiologically limited microenvironment that supports stem cells has been termed the "niche" and is generally used to describe the cellular components of the microenvironment surrounding stem cells as well as the interacting signals from the support cells [7,8]. The stem cell niche was first hypothesized by Schofield in 1978 and subsequently supported by various coculture experiments *in vitro* and by bone marrow transplantation [7]. Although these studies provided supportive evidence towards the niche theory, they did not describe the exact structure of the stem cell niche *in vivo*. Due to the difficulty in identifying and characterizing stem cell niches in mammals, the *Drosophila* and *C. elegans* model systems have been used to study the stem cell niches in *Drosophila* ovary and testis and the germ line stem cell niche in *C. elegans* [7,8]. The research in these genetic model systems has consequently lead to a better understanding of mammalian hematopoietic, epithelial, intestinal, and neural stem cell niches with respect to the physical contacts and diffusible factors involved in niche organization and regulation [7,8]. Studies of stem cell niches in *Drosophila* and *C. elegans* as well as

mammalian tissues have lead to common features, structures, and functions that characterize the stem cell niche. The stem cell niche comprises of a group of cells in a special tissue location for the maintenance of stem cells, functions as a physical anchor by providing adhesion molecules such as integrin, generates extrinsic factors that control stem cell fate and number, and exhibits an asymmetric structure such that after cell division, one daughter cell is maintained in the niche while the other one leaves the niche and becomes a functionally mature cell [7]. Thus, recent studies have resulted in significant progress in establishing the fundamental principles of the stem cell niche and further investigation of the niche's cellular and molecular components will provide important insights for identification of the stem cell niche in different systems. Furthermore, the ability to recreate the stem cell niches *in vitro* will allow for the better understanding of the maintenance and expansion of stem cells as well as their therapeutic applications to human degenerative diseases since the efficiency of future stem cell transplantation will come to rely on culturing the transplanted cells to a state as similar as possible to the state of the stem cells found *in vivo*.

In order to investigate these stem cell niches *in vitro*, it is important to design three-dimensional microenvironments that mimic the microenvironments of stem cells *in vivo* [9,10]. The behavior and differentiation patterns of stem cells as they occur within the body can only be best understood when researched under similar conditions of signal molecule gradients. Therefore, to study stem cells in their proper niches, an artificial, engineered microenvironment is needed that allows for the construction of the stem cell microenvironment and observation of the cells through a time-course in order to study their behavior [9]. Thus, the field of biomaterials engineering is an important contributor in the development of stem cell research since both disciplines of engineering and stem cell biology are needed to implement an artificial

stem cell environment. Although the complexity of the stem cell niche is challenging to reproduce, a number of biosynthetic technologies have been developed for stem cell culture that mimic cell-cell and cell-matrix interactions and modulate stem cell self-renewal and differentiation characteristic of stem cell niches [2,9-14].

In designing an engineered microenvironment, several stem cell niche factors must be taken into consideration including but not limited to cell-cell interactions, cell-matrix interactions, immobilized growth factors, matrix stiffness, topography, oxygen gradients, and patterning cells and ligands [11,12]. Studies have shown that fibronectin plays an important role in stem cell adhesion and interactions with the extracellular matrix, which provide key signaling molecules and ligands that lead to greater stem cell expansion [11]. Furthermore, murine embryonic stem cells were shown to expand with greater efficiency while maintaining differentiation potential on electrospun polyamide nanofibers that created an artificial meshwork upon which the cells could interact more efficiently [11]. Various synthetic collagen-coated polyacrylamide gels of different degrees of stiffness have caused different differentiation patterns on human mesenchymal stem cells [2,13]. In addition, Park *et al*. have developed oxidative microgradients to control pO_2 levels, which is important since stem cell niches are sensitive to pO_2 and will only differentiate and proliferate under specific pO_2 conditions [11]. Cell patterning and ligand concentration gradients have also been shown to induce several differentiation fates in a single culture, indicating the need for patterned ligand concentration gradients in accurately mimicking stem cell niches [2,11].

Previous experiments have established two-dimensional microenvironments making use of Transwell chambers that relied solely on simple diffusion of cells from one side of the membrane to the other [14]. However, these devices are extremely limited in establishing stable

concentration gradients and only allow for the quantification of the number of cells that migrate towards the ligand-containing medium. Thus, three-dimensional microfluidic devices have recently become of more interest to stem cell biologists as the fluid flow in these devices accurately mimic vasculature *in vivo* and provide control over the soluble and mechanical parameters of the cell culture environment [10]. Originating from the microfabrication technology of the electronic industry, microfluidic devices make use of microcapillaries that match the size of cell and blood capillaries [10]. The most common material used for microfluidic devices is poly-dimethylsiloxane (PDMS), which is soft, transparent, permeable to gasses, impermeable to liquids, biocompatible, nontoxic, and has a low electrical conductivity [10,14]. The flow in these devices is established by pressure-driven syringe pumps and with the continuous perfusion, the outlets of the device remove metabolic waste whereas the inlets provide fresh medium with nutrients and oxygen [10]. These microfluidic devices are ideal for stem cell niches in that they allow for the establishment of gradients of the soluble environment and control of mechanical forces that contribute to stem cell self-renewal and differentiation. Ong *et al*. have developed a microfluidic device containing a central compartment for cell culture and two side inlets for medium profusion (Figure 1); however, although this transparent system allows for imaging of cell behavior in response to ligand concentration gradients, the use of only one inlet and outlet on opposite sides of the device forces the medium to in a unidirectional path, creating more mechanical shear stress that may not be representative of the cell environment *in vivo* [10]. Furthermore, Figallo *et al*. have developed more complex microbioreactors and microfluidic systems (Figure 2), but these devices have similar problems and limitations as the device developed by Ong *et al* [9].

The most promising technology in the development of artificial microenvironments has been the recent design of a microfluidic device with minimal fluid shear stress as proposed by Shamloo *et al* (Figure 3) at Stanford's materials science and engineering department [14]. This artificial microfluidic device contains source channels in which cell media with a given ligand can be injected and sink channels in which cell media without ligand can be injected so as to create a stable gradient of ligand within the cell chamber where the cells can be injected. Overall, the innovative aspect of this engineered microenvironment is that it creates a stable concentration gradient and minimizes fluid convection within the cell culture chamber through the use of microcapillaries. Furthermore, this device addresses the limitations of previous devices in creating a three-dimensional substrate in which cell behavior can be observed with continuous flow even in non-adherent cultures. Another important feature of this device is that it allows for precise quantification of the ligand concentration gradient needed to induce various behaviors such as cell chemotaxis and cytoskeleton rearrangement. Although the cells used in this experiment were human umbilical vein endothelial cells [14], the innovative technology presented in this engineered device indicates a number of future experiments in better understanding the stem cell niche for a variety of stem cell populations. Single cell tracking, time-lapse imaging, and fluorescent imaging of cells expressing enhanced green fluorescent protein (eGFP) could be easily done using this transparent microfluidic device that provides a clear imaging interface in which cells can be viewed directly with a light microscope [14]. This device can also be easily modified to study three-dimensional migration through hydrogels loaded into the cell chamber, which would further mimic the cellular environment *in vivo* and allow for the actual transplantation of the stem cells in their optimal niche. Furthermore, this device can also be altered to incorporate an array of competing concentration gradients to study

the potential competition or synergy between multiple factors. Due to the flexibility of this microfluidic device in accurately engineering stem cell niches, the exact molecular conditions and behavior of cells in the niche can be better understood which in turn will lead to the identification of optimal conditions for the most effective stem cell transplantation [14].

Biomaterial-based scaffolds have been the most important tool for stem cell tissue transplantation by providing a three-dimensional cell environment representative of stem cell niches *in vivo*, which enhances cell differentiation, proliferation, attachment, and organization [2,13]. Biomaterials research has provided a variety of natural and synthetic materials that can be easily modified for the development of scaffolds representative of the three-dimensional cellular environment. Natural biomaterials for scaffolds consist of extracellular matrix components including collagen, fibrinogen, hyaluronic acid, glycosaminoglycans, and hydroxyapatite [2]. The major drawback with these materials is that the degradation rates of these materials cannot be easily controlled and because these are natural materials, interactions between the cells and scaffold cannot be easily predicted. Thus, synthetic biomaterials such as polyglycolic acid, polyactic acid, and copolymer polyactide-*co*-glycolide have been used as three-dimensional scaffold materials for evaluating cell behavior [2]. Perhaps the most attractive biomaterial used in scaffolds is synthetic hydrogels, which resemble the consistency of soft, native tissues and can be modified with hyaluronic acid to increase the modulus of elasticity [2,9,12,13]. The advantage of hydrogels is that they could be directly injected into tissues whereas other scaffolds must be surgically transplanted [13]. Furthermore, nanotechnology allows for the inclusion of nanoparticles that would enclose degradation molecules in the three-dimensional scaffolds, which would allow for the stem cells to differentiate and proliferate to optimal conditions before being exposed to the surrounding tissue *in vivo* [13]. The development

of biomaterials for three-dimensional scaffolds will play an important role in the future of stem cell transplantation in providing a mechanism to deliver the specific stem cell culture *in vivo*. However, it is perhaps even more important to first fully understand specific stem cell niches and the optimal conditions for stem cell renewal and differentiation by using the technology of microenvironments and then apply these findings to a scaffold for transplantation.

The transition from three-dimensional microenvironments to actual implantation of the cultured stem cell niche will require a biodegradable material to encapsulate the stem cell niche and to insure an immune response is not triggered [12]. Furthermore, innervation poses a problem as cells of organs are influenced by the parasympathetic and sympathetic nervous system and thus in terms of large tissue transplantation, the stem cell niche will require the development of nerves as well [13]. In addition, organ systems are composed of multiple cell types and thus transplanted hydrogels containing stem cell niches will have to be accommodate for more than one type of cell either by implanting multiple stem cell niches specific to each cell type or by creating subenvironments within the engineered niche to allow for the development of multiple cell types [13]. Although there may be no definitive solution as of right now to the problems posed by transplanting these artificial stem cell niches, research in the field of tissue engineering will elucidate answers to these problems as three-dimensional engineered microenvironments allow for a better understanding of specific stem cell niche behavior and function.

Given a new era of restoring scientific integrity by making scientific decisions based on facts rather than ideology, stem cell research will become even more important in providing the therapeutic answers to a wide variety of diseases. Thus, in order to insure that stem cell research is conducted in its most natural environment, it is important to use three-dimensional, engineered

microenvironments that would allow for the gradients of signaling molecules as well as cell-cell interactions. Although there has been extensive research on the identification of mammalian stem cell niches [7,8], these niches need to be further investigated in an environment that parallels the conditions of stem cell niches *in vivo*. The field of biomaterials engineering has already developed a variety of technologies to allow accurate replications of stem cell niches and even though most of them have some limitations, some of these microenvironment technologies seem to hold great promise, especially the microfluidic device proposed by Shamloo *et al* [2,9,12-14]. Thus, with the merger of the two great disciplines of biomaterials engineering and stem cell biology, a powerful therapeutic research tool lies in front of us that has the potential of bringing us closer to the reality of treating a wide range of diseases with simply our own cells [13].

Appendix

Figure 1: Microfluidic device designed by Ong *et al.*

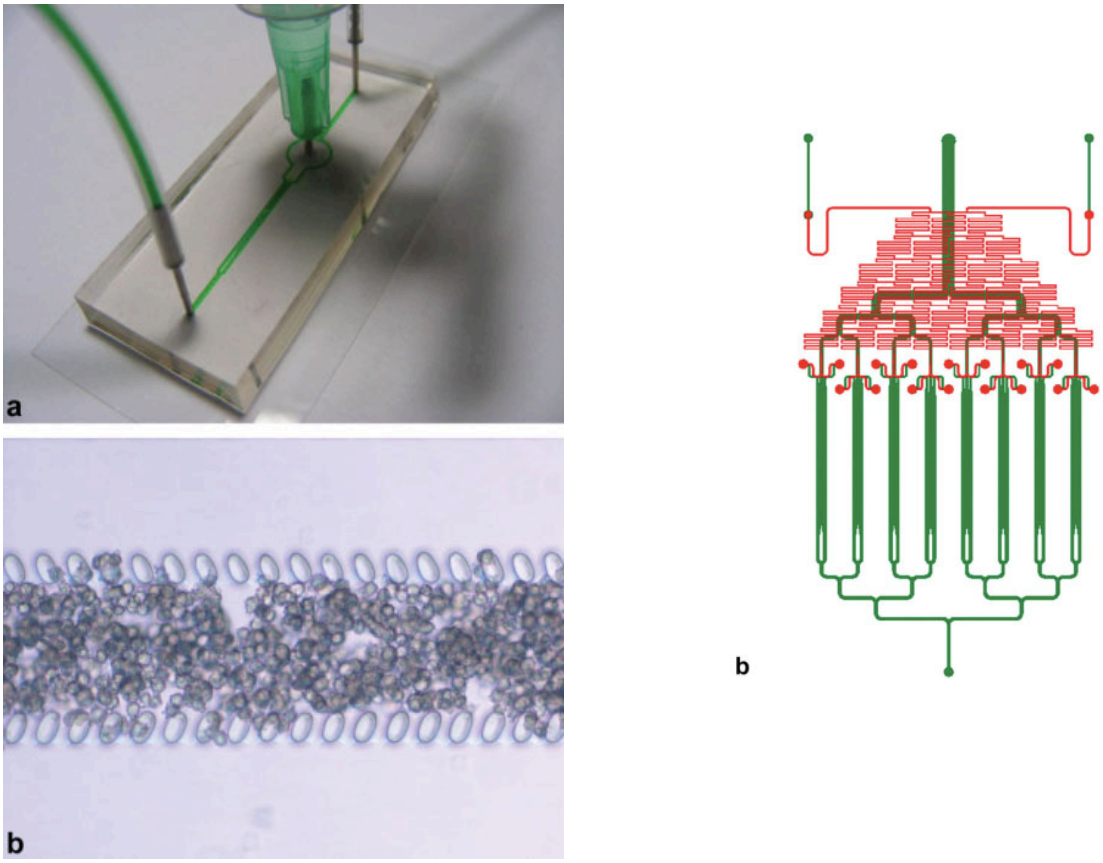


Figure 2: Microfluidic device developed by Figallo *et al.*

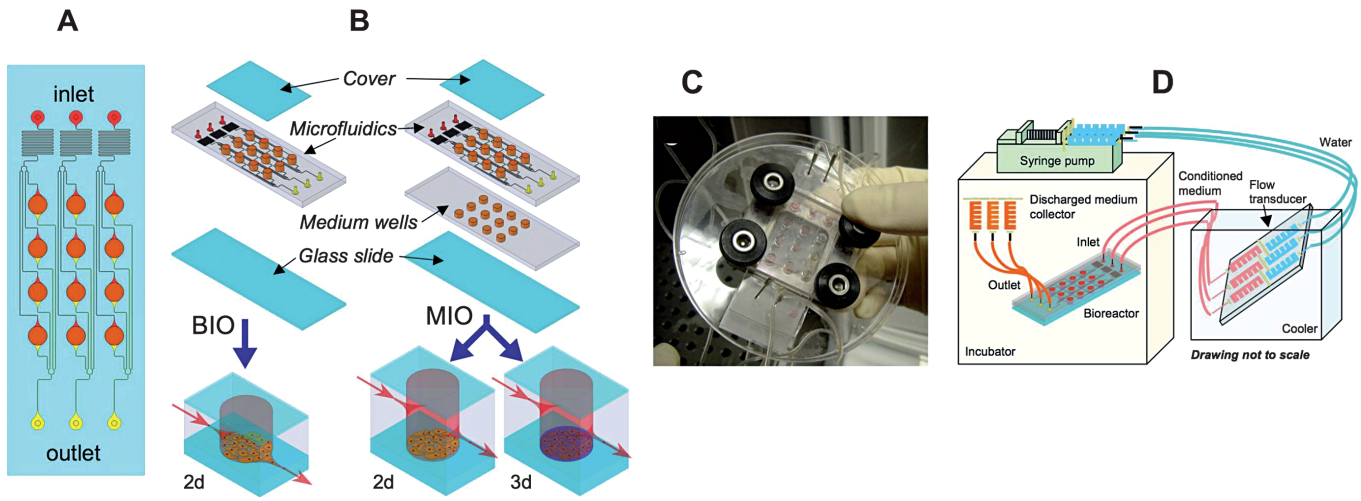
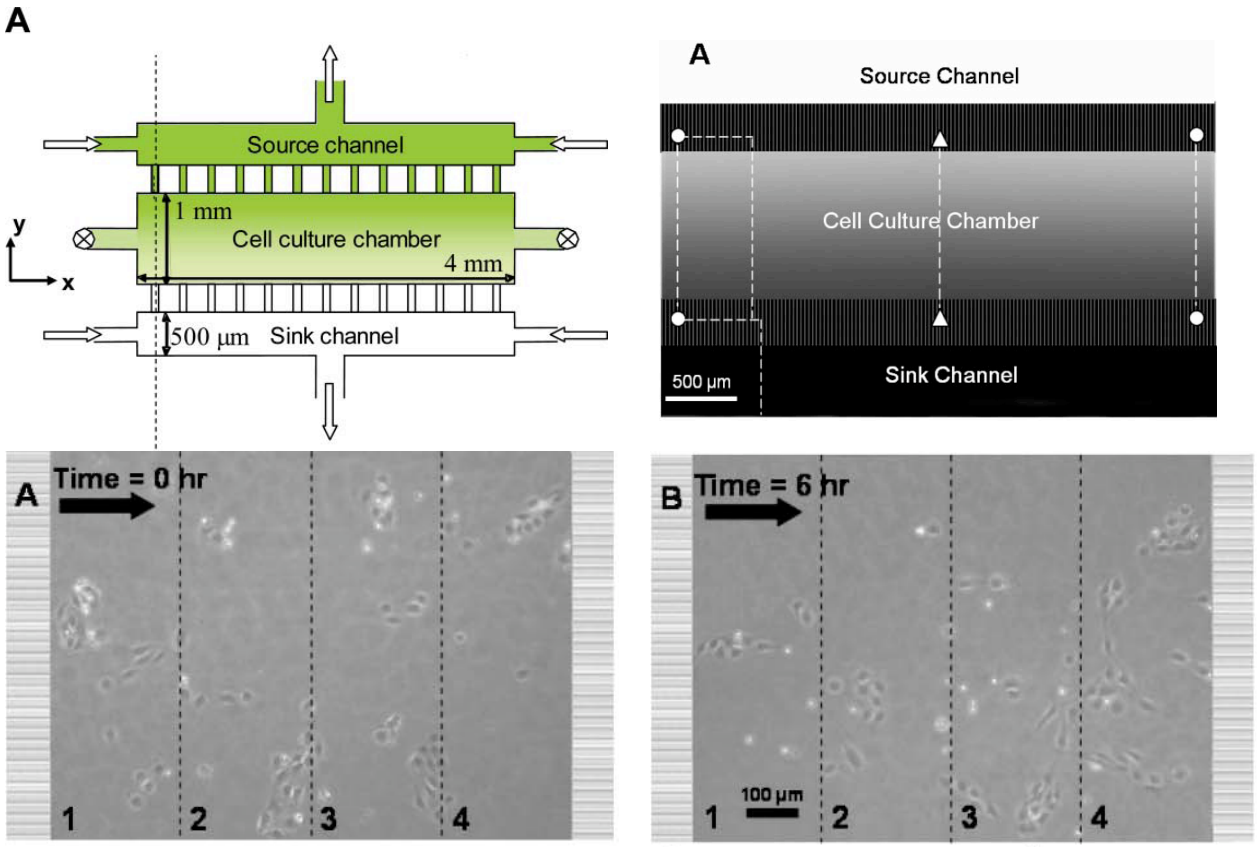


Figure 3: Schematic diagram and cell migration imaging of microfluidic device from Shamloo *et al.*



References

1. Singec I, Jandia R, Crain A, Nikkhah G, Snyder EY: **The Leading Edge of Stem Cell Therapeutics**. *Annu. Rev. Med.* 2007, **58**: 313-328.

2. Dawson E, Mapili G, Erickson K, Taqvi S, Roy K: **Biomaterials for stem cell differentiation**. *Advanced Drug Delivery Reviews* 2008, **60**: 215-228.

3. Adams GB, Scadden DT: **A niche opportunity for stem cell therapeutics**. *Gene Therapy* 2008, **15**: 96-99.

4. Daya SM, Watson A, Sharpe JR, Giledi O, Rowe A, Martin R, James SE: **Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction**. *Opthamology* 2005, **112.3**: 470-477.

5. Macchiarini P, Jungelbluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, Martorell J, Bellini S, Parnigotto PP, Dickinson SC, Hollander AP, Mantero S, Conconi MT, Birchall MA: **Clinical Transplantation of a tissue-engineered airway**. *The Lancet* 2008, **372.9655**: 2023-2030.

6. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S: **Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors**. *Cell* 2007, **131**: 861-872.

7. Li L, Xie T: **Stem Cell Niche: Structure and Function**. *Annu. Rev. Cell Dev. Biol.* 2005, **21**: 605-631.

8. Walker MR, Patel KK, Stappenbeck TS: **The stem cell niche**. *J. Pathol.* 2009, **217**: 169-180.

9. Burdick JA, Vunjak-Novakovic G: **Engineered Microenvironments for Controlled Stem Cell Differentiation**. *Tissue Engineering Part A* 2009, **15.2**: 205-219.

10. Noort DV, Ong SM, Zhang C, Zhang S, Arooz T, Yu H: **Stem Cells in Microfluidics**. *Biotechnol. Prog.* 2009, **25.1**: 52-60.

11. Dellatore SM, Garcia AS, Miller WM: **Mimicking stem cell niches to increase stem cell expansion**. *Current Opinion in Biotechnology* 2008, **19**: 534-540.

12. Godier A, Marolt D, Gerecht S, Tajnsek U, Martens TP, Vunjak-Novakovic G: **Engineered Microenvironments for Human Stem Cells**. *Birth Defects Research (Part C)* 2008, **84**: 335-347.

13. Chai C, Leong KW: **Biomaterials Approach to Expand and Direct Differentiation of Stem Cells**. *Molecular Therapy* 2007, **15**: 467-480.

14. Shamloo A, Ma N, Poo M, Sohn LL, Heilshorn SC: **Endothelial cell polarization and chemotaxis in a microfluidc device**. *Lab Chip* 2008, **8**: 1292-1299.